

## CLAIMS

- 5 1. A method of normalizing products of reverse transcription reaction of mRNA extracted from different biological samples, wherein said method comprises the steps consisting of
- a) adding a synthetic messenger RNA (SmRNA) which does not compete with the reverse transcription of target mRNAs of said sample and which do not interfere
- 10 with the reverse transcription of endogenous mRNA of said sample to a reaction mixture comprising mRNA extracted from said sample;
- b) determining the reverse transcription efficacy  $\rho$  for the smRNA and,
- c) adjusting the level of target mRNA transcripts by multiplication with  $\rho$ .
- 15 2. The method of claim 1, wherein a SmRNA displaying less than 95%, 97% or 99% or preferably less than 99,50 % identity with any mRNA of said sample, is used to perform a non-competitive reverse transcription reaction and to calibrate the quantity of each target mRNA obtained after amplification with the quantity of the cDNA amplified corresponding to said SmRNA.
- 20 3. The method of claim 1, wherein a SmRNA unable to hybridize under stringent conditions with any mRNA of said sample, is used to perform a non-competitive reverse transcription reaction and to calibrate the quantity of each target mRNA obtained after amplification with the quantity of the cDNA amplified corresponding
- 25 to said SmRNA.
4. The method of claim 1, wherein the SmRNA comprise a segment which is homologous to the target mRNA and is devoid of sequences complementary to the primers sequences used to amplify the target mRNA.
- 30 5. The method according one of claims 1 to 4, wherein said SmRNA is added in different reaction mixtures comprising one sample in different dilutions.

6. The method according to one of claim 1 to 5, wherein said SmRNA further comprises a polyA segment.
7. The method according to one of claims 1 to 6, wherein said SmRNA is about 80  
5 nucleotide long or longer, preferably about 100 nucleotide long.
8. The method according to one of claims 1 to 7, wherein said SmRNA is specifically amplified with a pair of primers designed to avoid primer dimerization.
- 10 9. A method according to one of claims 1 to 8 consisting of non-competitive quantitative RT-PCR, wherein quantification of target mRNAs is performed by means of normalization with said SmRNA.
10. A method according to one of claims 1 to 4 consisting of non-competitive DD-  
15 RT-PCR, wherein quantification of target mRNAs is performed by means of normalization with said SmRNA.
11. A method according to one of claims 9 and 10 which is performed on DNA  
microarrays.
- 20 12. A method according to claim 11, wherein cDNA (ScDNA) corresponding to the SmRNA is amplified and spotted onto said microarrays.
13. A method according to one of claims 1 to 11 consisting of Northern blotting  
25 wherein said SmRNA is pooled with samples.
14. A method according to one of claims 1 to 13, wherein said SmRNA consists of a SEQ ID No 1 or SEQ ID No 2 or sequences deriving thereof.
- 30 15. A method according to claim 14, wherein said SmRNA is reversed transcribed into cDNA of SEQ ID No 3 using a pair of primer consisting of SEQ ID No 4 and SEQ ID No 5.

16. A synthetic messenger RNA (SmRNA) designed for normalization of reverse transcription reaction of mRNAs of a biological sample, wherein said SmRNA does not compete with target mRNAs and does not interfere with the reverse transcription of endogenous mRNA of said sample, which SmRNA displays less than 95% or 99% identity with any biological sequence of said sample or does not hybridize under stringent conditions to any with any biological sequence of said sample, comprises a poly A segment and is about 80 nucleotide long or longer, preferably about 100 nucleotide long.
17. A SmRNA according to claim 16 which further comprise at least two segments which are complementary to at least two primers that are designed to avoid primer dimerization.
18. A SmRNA according to one of claims 16 and 17 consisting of SEQ ID No 1 or SEQ ID No 2.
19. A SmRNA according to claim 17 comprising two segments which are complementary to at least two primers of SEQ ID No 5 and SEQ ID No 6.
20. A primer or probe selected from of SEQ ID No 5 and SEQ ID No 6.
21. A cDNA obtained from a reverse transcription reaction of a SmRNA according to one of claims 16 to 19.
22. A cDNA according to claim 21 which consist of SEQ ID No 3 or SEQ ID No 4.
23. A vector, more particularly a plasmid, comprising a sequence encoding the SmRNA of one of claims 16 to 19.
24. A vector according to claim 23 which further comprise any sequence corresponding to SEQ ID No 9, 10 and 11 preceded by a sequence corresponding to a RNA polymerase promoter, in particular RNA polymerase T7 promoter (sequence ID No 8) and referred as to "DNA probe DNA $\Sigma$ " (see figure 3).

25. A vector according to claim 23 or 24 which comprises the construct as shown in figure 1, notably the sequence SED ID No 7 or SEQ ID No 11.
- 5 26. A vector according to claim 25 as depicted in figure 2.
27. A kit for quantification of mRNAs of a biological sample comprising a SmRNA according to one of claims 16 to 19, or a vector according to one of claims 19 to 22.
- 10 28. A kit according to claim 27 further comprising primers of SEQ ID No 5 and 6.
29. The use of a SmRNA according to one of claims 16 to 19 or a vector according to one of claims 23 to 26 for calibrating target mRNAs during quantification in RT-PCR reaction.
- 15 30. The use according to claim 29 in Q-RT-PCR or DD-RT-PCR.
31. The use according to claims 29 or 30 in frame with DNA microarrays.
- 20 32. The use of a SmRNA according to one of claims 16 to 19 or a vector according to one of claims 23 to 26 for calibrating target mRNAs in Northern blot analysis and for calibrating antisense RNA amplification method.